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Predator–Prey Battle of Ecological Icons: Horned Lizards (*Phrynosoma* spp.) and Harvester Ants (*Pogonomyrmex* spp.)

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Horned lizards, *Phrynosoma* spp., and harvester ants, *Pogonomyrmex* spp., could be in a predator–prey arms race in which the lizards are specialists that feed on harvester ants, and ants have highly toxic venom and other defenses to help deter predacious horned lizards. All 23 examined species of harvester ants possess venoms that are highly lethal to mice, but the venoms of the tested ant species were nearly inactive toward horned lizards. Blood plasma of *Phrynosoma cornutum* contains a factor (or factors) that neutralizes the ability of harvester ant venom to kill mice, but does not neutralize the venoms of honeybees, a rattlesnake, Russell's viper, or a cobra. A species of harvester ant present only in southern South America was used to test the predictions that the lethality of harvester ant venom evolved in response to predation pressure from horned lizards, and that horned lizard plasma does not neutralize the lethality of this species of harvester ant. This ant species did not overlap in range with horned lizards, which have a range from Guatemala to Canada. Not only was the venom of the South American ant species the most lethal of all tested harvester ant venoms, the venom's lethal activity was neutralized by horned lizard plasma. These results indicate that horned lizards responded to the lethality of their invertebrate prey's venom, but that the harvester ant venom lethality did not evolve in response to predation pressures by present day horned lizards.

Las lagartijas cornudas, *Phrynosoma* spp., y las hormigas cosechadoras, *Pogonomyrmex* spp., parecen estar en una regata depredador-presa en la que los lagartos son especialistas que se alimentan de hormigas cosechadoras, y las hormigas tienen venenos altamente tóxicos y otras defensas para ayudar a disuadir a los depredadores lagartos cornudos. Se encontró que veintitrés especies de hormigas cosechadoras poseen venenos altamente letales para los ratones, pero los venenos de las especies de hormigas probadas estaban casi inactivos hacia los lagartos cornudos. El plasma sanguíneo de *Phrynosoma cornutum* contiene un factor (o factores) que neutraliza la capacidad del veneno de la hormiga recolectora para matar ratones, pero no neutraliza los venenos de las abejas, la serpiente de cascabel, la víbora de Russell, o la cobra. Una especie de hormiga cosechadora presente solo en el sur de Sudamérica se usó para probar las predicciones de que la letalidad del veneno de la hormiga cosechadora evolucionó en respuesta a la presión de depredación de los lagartos cornudos, y que el plasma del lagarto cornudo no neutraliza la letalidad de esta especie de hormiga cosechadora. Ni la especie de hormiga ni su antepasado postulado se habían superpuesto nunca con las lagartijas cornudas, que tienen un rango desde Guatemala hasta Canadá. No solo el veneno de las especies de hormigas sudamericanas fue el más letal de todos los cosechadores y venenos probados, sino que la actividad letal del veneno fue neutralizada por el plasma de lagarto cornudo. Estos resultados indican que los lagartos cornudos respondieron a la letalidad del veneno de sus presas invertebradas, pero que la letalidad del veneno de la hormiga recolectora no evolucionó en respuesta a las presiones de depredación de los lagartos cornudos actuales.

HORNED lizards (*Phrynosoma* spp.) are flat, wide-bodied, cryptic, slow-running lizards inhabiting arid and semi-arid regions of North America from Western Canada and the USA to Guatemala (Sherbrooke, 2003). The genus currently consists of 17 generally recognized species with perhaps a number of undescribed species (Leaché and Linkem, 2015; Montanucci, 2015; Blair and Bryson, 2017) that feed primarily on small arthropods, with ants being the major component of the diet of many species. The Texas Horned Lizard, *Phrynosoma cornutum*, is a large species with the greatest geographical range of any of its congeners in the US. It is a dietary specialist feeding mainly on ants, with harvester ants (*Pogonomyrmex* spp.) being the preferred ant prey. Harvester ants are a low nutrient density diet (Porter and Jorgensen, 1981), and, hence, the lizards have enormous stomachs to handle the required volume of ants needed to sustain themselves (Pianka and Parker, 1975). The genus *Phrynosoma* originated in southern Mexico and radiated from there to its current limits within North America (Leaché and McGuire, 2006).

Harvester ants are abundant in many arid and semiarid regions of the New World, are relatively large ants (6 to 12 mm in length), and often construct visible cleared areas around their nests. The nests of many species of harvester ants are perennial and remain many years in the same location, and nests of the more prominent species contain thousands of individuals. Harvester ants consist of about 70 species in the genus *Pogonomyrmex,* plus three species in the genus *Patagonomyrmex* restricted to southern Argentina and Chile (Johnson and Moreau, 2016). In previous reports, the genus *Pogonomyrmex* has sometimes been divided into the subgenera *Pogonomyrmex* and *Ephebomyrmex*, but recently that division has been shown be invalid (Johnson and Moreau, 2016) and will not be considered here. Harvester ants originated about 60 million years ago in northern South America (Taber, 1998). From there, waves of speciation went south into the southern South America and north into North America, with two species reaching the Caribbean island of Hispaniola. At present, no harvester ant species are present in the Central American countries, with the exception of Guatemala.

All taxa of harvester ants are famous for their ability to deliver excruciatingly painful and long-lasting stings. Harvester ant venom is the most toxic ant, wasp, or bee venom known. Some species in the informal previously defined *californicus* group of Cole (1968) are also known to sting autotomize, that is, leave their stinger in the flesh of a person, a feature that likely prolongs venom delivery into the target after the ant has been removed (Schmidt, 2016). In this paper the harvester ants will be broken into three categories:

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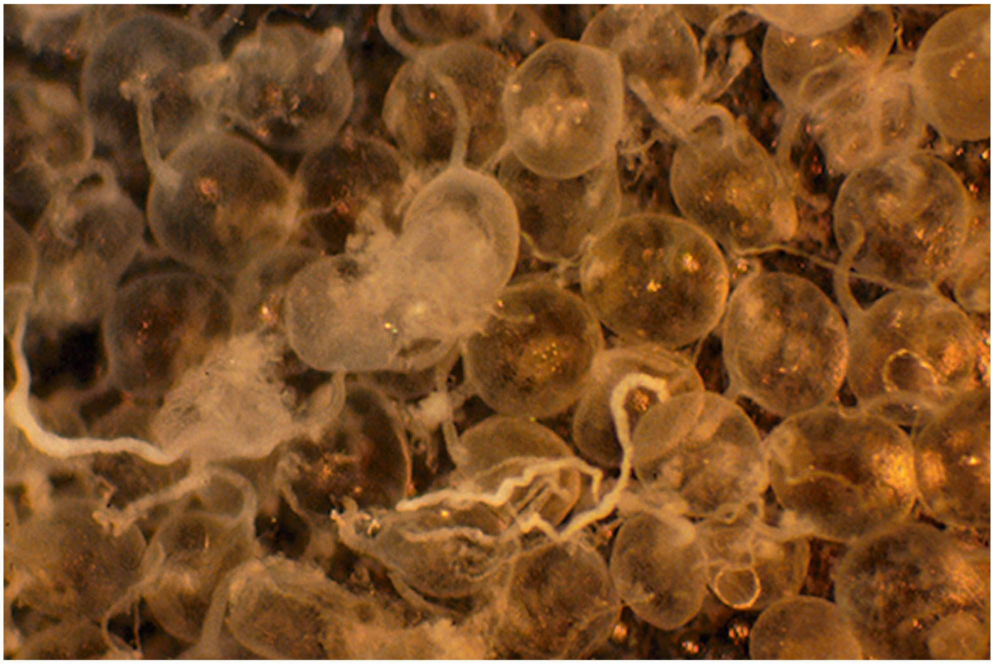

Fig. 1. Harvester ant venom reservoirs dissected from ants and ready for draining of venom.

those in the revised *californicus* group (*sensu* Johnson and Moreau, 2016); those North American species outside the *californicus* group; and the one available species from South America. The reason for distinguishing the *californicus* group from the others is that several species in this group (*P. californicus, P. maricopa, P. comanche,* and *P. badius*) are known to autotomize their sting apparatus into victims, a feature that might be important in defense. In humans, the venom also elicits unique sting symptoms including piloerection in which the hairs surrounding the sting site become erect and localized sweating in the same area (Schmidt and Blum, 1978). These latter two symptoms are consistent with the venom containing one or more neurotoxic components. The similarity of the feeling and reaction to the stings experienced during fieldwork on various species of harvester ants gave the impression that the venoms of the species within the genus were similar (pers. obs.). Supporting evidence for this included similar lethalities of the venoms of *P. badius, P. barbatus, P. comanche*, and *P. rugosus* (Schmidt et al., 1980) and that the venoms of all nine tested species of harvester ants were immunologically cross-reactive, indicating similarity or identity in the composition of the allergy-inducing venom components (Schmidt et al., 1984). Further testing of the hypothesis that the venoms are similar in activity across species of the genus was one goal of this investigation.

The venom from one harvester ant is sufficient kill a mouse, yet horned lizards eat harvester ants with impunity in quantities as great as 100 per day (Sherbrooke, 2003; Schmidt, 2016). Previous investigations revealed that the blood plasma of *Phrynosoma cornutum* was able to neutralize the toxicity of harvester ant venom (Schmidt and Schmidt, 1989; Schmidt et al., 1989). This observation led to speculation that horned lizards and harvester ants were engaged in a predator–prey arms race in which harvester ants evolved ever more toxic venoms and horned lizards evolved ever greater resistance to the toxic effects of their harvester ant prey (Schmidt and Schmidt, 1989). At the time of those reports, the only available material from both taxa came from individuals that were sympatric, thus rendering further evidence on the question elusive. In the year 2000, venom from an allopatric species of harvester ant located in southern South America was collected. This provided an opportunity to test the hypothesis that harvester ant venom toxicity evolved in direct response to selection pressure by predacious horned lizards. Evidence relating to this hypothesis is reported here.

MATERIALS AND METHODS

Ants and venom collection.—Twenty-two North American species of harvester ants in the genus *Pogonomyrmex*, including one species of which two subspecies were sampled, were collected in the United States and Mexico. In addition, one species from South America was obtained. Collection locations and dates are listed in Appendix 1. Ants were frozen and maintained at –20°C until dissected for venom.

Pure venom was obtained by the method of Schmidt (1986). In brief, frozen ants were thawed, their sting apparatuses removed to a spot of distilled water, the venom reservoir (minus filamentous glands) was pinched off at the duct and removed from the rest of the sting apparatus, twice rinsed with distilled water, and placed in clean distilled water (Fig. 1). Up to 100 reservoirs were collected into an approximately 50 μl droplet of distilled water, after which the venom was squeezed from the reservoirs and the empty chitinous reservoirs were discarded. The pure venom was lyophilized and stored at –20°C until used.

Lizards and lizard plasma.—*Phrynosoma cornutum* were collected in the San Simon Valley of the southern Arizona–New Mexico border area during the months of July, August, and September 1984. Yarrow's Spiny Lizards (*Sceloporus jarrovii*) were collected in the mountains surrounding the San Simon Valley during the same time period and served as a control lizard source of plasma. A common king snake (*Lampropeltis getula*) was collected in Tucson, Arizona in May 1984 and served as a control reptile.

Plasma from the lizards was obtained from fresh blood collected by decapitating individuals and draining the blood into Tris-buffered saline (TBS; 0.85% NaCl, 0.01 M TRIS HCl, pH 7.2, 0.1 M citrate). Collected blood was refrigerated at 4°C for less than seven days prior to erythrocyte removal and analysis. Immediately after collection, the plasma samples were frozen and stored at –20°C until used. Plasma of *Lampropeltis getula* was collected via cardiac puncture and treated after collection in the same manner as for the lizards.

Lethality determinations.—For each analysis, the calculated and measured weight of venom was dissolved in 0.15 M NaCl saline and injected in the volume of 0.6% of the animal body weight. Injections were injected intraperitoneally (i.p.) into three groups of four horned lizards and intravenously (i.v.) into the tail veins of three or four groups of six 18–20 g Swiss white mice. Median lethal amount of venom to kill 50% of the individuals (LD_{50}) in 24 hr were calculated according to the rapid 50% endpoint method that interpolates between the 25% and 75% values to obtain the most stable and reliable 50% lethality value, as described by Reed and Muensch (1938). The method reduces the number of animals needed and the time necessary for obtaining an accurate measure. The precision of the 50% measure is indicated as 95% confidence intervals (CI) that are determined by the number of animals in each dose and the intervals between doses as detailed by Pizzi (1950).

Venom neutralization analyses.—The ability of the plasma from *Ph. cornutum* to neutralize lethal effects of venom of *P. maricopa* and *P. cunicularius* was determined by intravenous injection into mice of uniformly proportional amounts per gram weight of mouse of plasma mixed with the venom. The plasma plus venom mixture was incubated for 5–20 minutes at ambient temperature before injection. Mortality was determined at 24 hours and lethality was calculated as described above. The ability of lizard plasma to neutralize the lethality of harvester ant venom was calculated as:

$$ED_{50} = \mu l \text{ plasma}/(xLD_{50} - 1LD_{50}),$$

where ED_{50} is the median effective dose, x equals number of times the LD_{50} amount of venom that was administered, and the LD_{50} is in units of µg/g. In essence, this calculation is based upon the inactivation as a complex of all the venom in excess of one LD_{50}, thereby showing the effect of the one LD_{50} amount of venom that was not neutralized and, in turn, caused the death of the animal. From this we can determine the amount of plasma required to neutralize the activity of the excess of venom above an LD_{50} that was injected.

RESULTS

The lethality of the venom for the 23 taxa of harvester ants averaged 0.61 mg/kg with a range from 0.088 to 1.51 mg/kg (Table 1). The three groupings, the *californicus* group known for sting autotomy, the rest of the North American species that do not have sting autotomy, and *P. cunicularius* from South America were evaluated separately. The *californicus* group was distinguished from the others because the venoms of these sting autotomizing species might be more lethal than venoms of the other species of harvester ants. If so, the loss of life of the individual autotomizing ant might be offset by the increased venom effectiveness of the sting apparatus remaining within the victim and delivering more of its venom. The *californicus* group had a mean lethality of 0.43 mg/kg compared to the rest (excluding *P. cunicularius*) of 0.71 mg/kg, a difference not significant ($P = 0.149$, t-test) and indicating that sting autotomy is not likely the result of increased venom toxicity of this group of species. The most lethal of the venoms is that of *P. cunicularius*, the South American species.

Table 1. Lethality of *Pogonomyrmex* harvester ant venoms to mice. Determinations by intravenous injection except *P. badius* by intraperitoneal injection. All species are from North America except *P. cunicularius* which inhabits only southern South America. Lethalities and their 95% confidence intervals listed for individual taxa; means and standard deviations listed for groups. The *californicus* species group is listed and evaluated separately because members of this species group are known to autotomize their stings into the target organism.

Ant species	Lethality (mg/kg)	95% Conf. interval (mg/kg)
North American *Pogonomyrmex* excluding *californicus* group		
P. bicolor	0.125	0.065–0.244
P. texanus	0.26	0.19–0.36
P. subdentatus	0.3	0.19–0.48
P. huachucanus	0.35	0.22–0.56
P. barbatus	0.39	0.21–0.71
P. wheeleri	0.42	0.30–0.58
P. tenuispina	0.48	0.35–0.67
P. montanus	0.63	0.44–0.89
P. apache	0.67	0.37–1.23
P. brevispinosus	0.74	0.52–1.04
P. rugosus	0.76	0.45–1.26
P. salinus	1.06	0.58–1.91
P. occidentalis	1.11	0.84–1.45
P. subnitidus	1.11	0.82–1.49
P. desertorum	1.4	0.99–1.99
P. salinus owyheei	1.51	0.88–2.60
Mean group lethality	**0.71**	**0.42 (s. dev.)**
californicus group		
P. maricopa	0.12	0.09–0.16
P. anzensis	0.22	0.11–0.41
P. badius	0.42	0.31–0.58
P. comanche	0.53	0.33–0.84
P. californicus	0.6	0.43–0.84
P. magnacanthus	0.71	0.49–1.03
Mean group lethality	**0.43**	**0.23 (s. dev.)**
P. cunicularius	0.088	0.066–0.140
Grand mean	**0.61**	**0.40 (s. dev.)**

Lethality of harvester ant venom to horned lizards.—*Phrynosoma cornutum* is highly resistant to the effects of harvester ant venom. Mice as the target species for measuring venom lethality are assigned a relative resistance factor of 1. Based on this reference value, *Ph. cornutum* challenged with venom of *P. maricopa* is 1361 times more resistant than mice (Table 2). The venom of *P. cunicularius*, the South American species that does not overlap in range with any horned lizards, is 1.35 times more toxic to mice than the venom of *P. maricopa*, the next most toxic known harvester ant venom (Table 2).

Ability of blood plasmas to neutralize harvester ant venom.—The blood plasmas of *Ph. cornutum* and the two control reptiles, *Sceloporus jarrovii* and *Lampropeltis getula,* were compared for abilities to neutralize the lethal activity of the venom of *P. maricopa* (Table 3). Control mice injected with 2.3 or 2.5 times an LD_{50} dose of venom of *P. maricopa* all died, an indication of high venom activity. When mice were injected with the plasma of *Ph. cornutum* (2 µl of plasma/g of mouse), the animals exhibited no ill effects, demonstrating that the venom activity had been eliminated (Table 3). When mice were subjected to a 3.6 times in LD_{50} challenge of

Table 2. Lethality of harvester ant venoms to mice and horned lizards. A relative resistance factor of 1 is assigned to mice challenged with venom of *Pogonomyrmex maricopa*; the other factors are determined by dividing their respective lethalities by the lethality of 0.119 for venom of *P. maricopa*. The values for *P. maricopa* are from Schmidt et al. (1989).

Venom source	Target species	Lethality (mg/kg)	95% Conf. interval (mg/kg)	Rel. resistance factor
Pogonomyrmex maricopa	Mice	0.119	0.072–0.197	1
P. maricopa	*Ph. cornutum*	162	61–432	1361
P. cunicularius	Mice	0.088	0.066–0.140	0.74

venom of *P. maricopa* (0.43 mg/kg; Table 3) incubated in 0.83 μl of plasma of *Ph. cornutum*/g of mouse, all mice survived. Neither of the control plasmas from *S. jarrovii* or *L. getula* conferred any protection against effects of the venom of *P. maricopa* (Table 3).

Venom of *P. cunicularius* at 2.5 times an LD_{50} challenge killed all mice when administered alone, or with 0.283 μl plasma of *Ph. cornutum*/g of mouse (Table 3). The amount of plasma required to yield 50% protection against a dose of 2.5 times the LD_{50} of venom of *P. cunicularius* is 0.40 μl plasma/g of mouse with a 95% confidence interval of 0.29–0.55 μl plasma/g of mouse. At 5 times an LD_{50} dose of venom from *P. cunicularius*, five of six mice survived when the venom was incubated with 1.07 μl plasma/g of mouse. At this 5 times dose, the amount of plasma required to yield 50% protection is 1.23 μl plasma/g mouse with a 95% confidence interval of 0.95–1.59 μl/g. The effective dose (ED_{50}) of plasma that neutralizes 1 μg of venom of *P. cunicularius* when the dose level is 2.5 times the LD_{50} is 3.03 μl plasma/μg of venom and when 5 times the LD_{50}, the ED_{50} is 3.50 μl plasma/μg of venom.

DISCUSSION

Worker harvester ants have few specialist vertebrate predators that consume large numbers of individuals, though a variety invertebrates, especially spiders and some wasps, are known to take foraging harvester ants outside the nest (Schmidt and Schmidt, 1989; Schmidt, 2016). The exceptions to this absence of major predators are the horned lizards, many species of which have become specialized in feeding on harvester ants. The relationship between horned lizards and harvester ants has led to speculation that the two taxa are in a predator–prey arms race. This speculation raises several hypotheses: 1) the venoms of the various species of harvester ants are similar in their toxic activities; 2) horned lizards gained the ability to eat harvester ants, in part, because of their evolution of a venom detoxifying activity, thereby rendering them resistant to the strongest defense of the ants; and 3) the extraordinary venom toxicity of harvester ants evolved in response to predation by horned lizards or their ancestors, and that toxicity was maintained during their subsequent speciation.

The first hypothesis, that all species of harvester ants possess venoms with similar toxic activities, is generally supported. The reactions experienced to stings of different species of harvester ants are similar and produce similar pain and symptoms in humans (pers. obs.). Moreover, all 23 tested species of harvester ants have exquisitely lethal venom (Table 1), venoms that are more lethal than almost all other stinging insect venoms (Schmidt, 2014). Despite the most toxic known stinging insect venom being that of a species of harvester ant, this hypothesis is only partially supported because a 17-fold range of lethalities is observed among the taxa.

Once venom toxicity evolved in harvester ants, it appears to have been retained in at least two North American environments where it serves no benefit against horned lizards. One environment is on the hot south-facing slopes in Anza Borrego Park in California in which no horned lizard species exist, but the harvester ant, *P. anzensis,* does. In this extraordinary environment, the only environment in which *P. anzensis* exists, the species responded to the harshness not by losing venom lethality, but by sacrificing production of venom via a reduction by 80% of the amount of venom produced (Schmidt and Snelling, 2009). The second environment is the southeastern part of the United States where the species *P. badius* lives and no horned lizards or other important vertebrate predators are known to exist. This species of harvester ant is in a relatively biologically benign environment and exhibits no reduction in venom production or activity. The third example of venom toxicity being maintained in the absence of horned lizards is the South American species *P. cunicularius* that was collected in Argentina, yet had extraordinarily lethal venom.

Table 3. Ability of blood plasma of three species of reptiles to neutralize the lethal effects of harvester ant venom. Data for *P. maricopa* from Schmidt et al. (1989) and Schmidt and Schmidt (1989).

Venom	Venom (μg/g mouse)	Factor xLD_{50}	Plasma source	Plasma (μl/g mouse)	% Mortality (*n* animals)
P. maricopa	0.28	2.3	*Ph. cornutum*	0	100 (8)
P. maricopa	0	0	*Ph. cornutum*	2	0 (2)
P. maricopa	0.43	3.6	*Ph. cornutum*	0.83	0 (4)
P. cunicularius	0.22	2.5	*Ph. cornutum*	0	100 (6)
P. cunicularius	0.22	2.5	*Ph. cornutum*	0.283	100 (6)
P. cunicularius	0.22	2.5	*Ph. cornutum*	0.4	50 (6)
P. cunicularius	0.22	2.5	*Ph. cornutum*	0.566	0 (6)
P. cunicularius	0.44	5	*Ph. cornutum*	0	100 (6)
P. cunicularius	0.44	5	*Ph. cornutum*	0.75	100 (6)
P. cunicularius	0.44	5	*Ph. cornutum*	1.07	16.7 (6)
P. maricopa	0.33	2.8	*S. jarrovii*	3.14	100 (4)
P. maricopa	0.33	2.8	*L. getula*	1.67	100 (3)

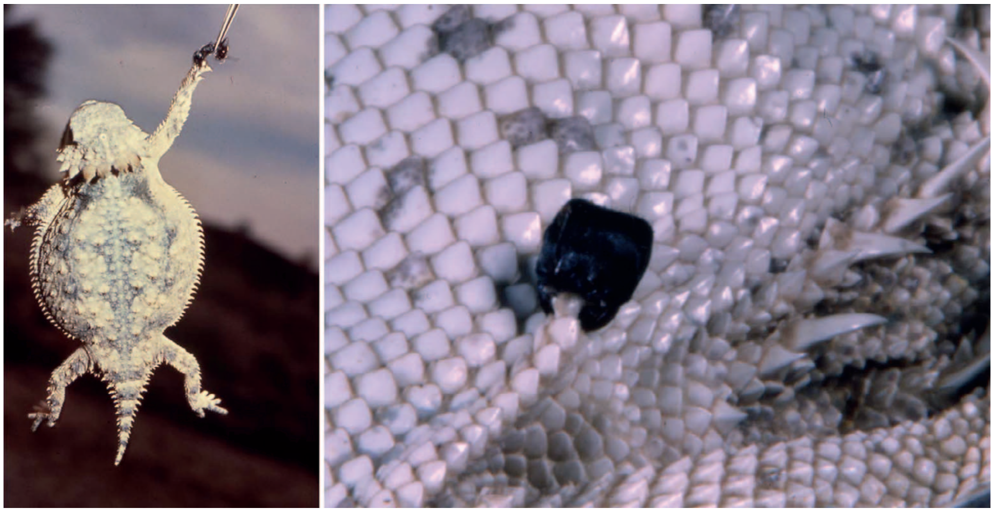

Fig. 2. *Phrynosoma solare* being lifted while being bitten by *Pogonomyrmex rugosus* (left panel); head of *Pogonomyrmex rugosus* remaining attached to belly scales of a horned lizard after the ant body had been broken off (right panel).

The second hypothesis, that horned lizards gained the ability to eat harvester ants because of their evolution of a detoxifying mechanism, is supported. *Phrynosoma cornutum* can withstand over 1350 times the lethal dose of harvester ant venom that kills mice. A similar resistance is also found in the horned lizard, *Ph. hernandesi,* whereas the related lizard species, *Sceloporus jarrovii,* a species that does not generally feed on harvester ants, is much less resistant (Schmidt and Schmidt, 1989). Direct evidence of the resistance of horned lizards to the venom toxicity of harvester ants is observed in the neutralization by horned lizard plasma of the lethal effects of venom plus plasma injected into mice. In contrast to the plasmas of horned lizards, the plasmas of *S. jarrovii* and *L. getula* do not neutralize harvester ant venom, an indication that these reptiles do not have protective factors against harvester ant venom. Even at five times the lethal challenge of venom, the plasma could protect the mice. The LD_{50} at five times the lethal dose of venom when combined with plasma indicates that the plasma effectively blocks the activity of $4LD_{50}$ doses, and only after the neutralizing ability of the plasma is exhausted does the remaining venom affect the mice. In essence, the plasma acts as a toxin-inactivating sponge that prevents the toxin from reaching its target and doing its damage. The plasma factor(s) that combines with venom toxin to inactivate the toxin is stable for a long period of time when stored at –20°C in the dark. This stability was demonstrated by a lack of activity loss after storage for 16 years (from 1984 until 2000) between its use with the venoms of *P. maricopa* and *P. cunicularius*.

The toxin inactivating factor in horned lizard plasma evolved in response to harvester ant venom, and not to venoms in general. This is shown by the inability of horned lizard plasma to neutralize the toxicity of the venoms of honeybees (*Apis mellifera*), a rattlesnake (*Crotalus adamanteus*), Russell's viper (*Vipera russelli*), or a cobra (*Naja naja atra*; Schmidt and Schmidt, 1989). The specific neutralization of harvester ant venom activity does not mean that the predator–prey arms race hinged only on the defeat of the harvester ants' strongest defense. In addition to being able to neutralize the toxic effects of harvester ant venom, horned lizards produce a mucous that coats ingested ants and mostly renders them incapable of stinging the digestive tract of the lizard (Sherbrooke and Schwenk, 2008). For their part, harvester ants are not defenseless against horned lizards—they possess powerful mandibles that can effectively bite and possibly injure horned lizards (Fig. 2). During such biting events, a lizard often flees from the ants (Sherbrooke, 2003).

The third hypothesis, that the extraordinary venom toxicity of harvester ants evolved in response to predation by horned lizards or their ancestors and was maintained during their subsequent speciation, is not supported. Horned lizards evolved in southern Mexico and from there species radiated to Guatemala in the South and to the western provinces of Canada in the north. They are currently not found in Central or South America (Leaché and McGuire, 2006). Harvester ants originated from an ancestor in northern South America and radiated from there southward into Argentina and Chile, and northward into Western Canada. No species are currently present in Central America except in Guatemala (Tabor, 1998; Johnson and Moreau, 2016). The ranges of the ancestors of harvester ants and horned lizards did not overlap, and modern-day horned lizards are not known from the region of the harvester ant ancestor. Therefore, the South American species of harvester ants never experienced horned lizards, though their ancestors might have experienced some type of ancestor of horned lizards. *Pogonomyrmex cunicularius* is only present in southern Brazil through northern Argentina, indicating that the species did not experience direct selection pressure from horned lizards. Despite this, the species has the most lethal venom of any harvester ant, something that had to evolve in response to pressure other than that exerted by horned lizards as we currently know them. I am unaware of any literature or expert observation of any lizards, or other likely vertebrate predators, in the range of *P. cunicularius* that could have affected the evolution of the venom of the species. The implications of this are that the extreme venom toxicity of *P. cunicularius* must have originated with an ancestral harvester ant species in response to unknown pressures.

Dual predictions relating to the venom of *P. cunicularius* are that the venom should be less lethal than that of North American species of harvester ants, and that the plasma of horned lizards should not be able to neutralize the venom toxicity of the species. Neither hypothesis is supported because *P. cunicularius* is the most toxic of all harvester ant venoms, and because its venom lethality is neutralized by horned lizard plasma. The causes of the extreme lethality of harvester ant venoms appears not to have been the result of direct predation by horned lizards. Furthermore, why the venom of *P. cunicularius* is neutralized by horned lizard plasma is a mystery. One possible explanation to why *P.*

cunicularius has such lethal venom is that its common ancestor might have dispersed northward into the range of horned lizards (or their common ancestors) and experienced predation there before subsequently migrating south again and retaining its venom lethality. Another possible explanation is the reverse situation—that is, horned lizards, or their common ancestor, might have migrated southward into the range of the ancestor of harvester ants and then either migrated back northward, or became extinct in its southern range and we have no fossil record of them.

A final unknown in the biology of harvester ants is the impact of potential seed predators that raid the ants' seed stores. A variety of rodents, such as kangaroo rats (*Dipodomys* spp.), are known to be granivores that could utilize the major food reserves of the ants and, in doing so, indirectly and negatively influence the ants' ability to survive and reproduce. Whether or not rodents or other granivores were an important selection force on the venom lethality of harvester ants is an intriguing mystery that deserves attention.

In summary, the horned lizard–harvester ant relationship may not be a bilateral predator–prey arms race. Rather, the "race" appears to be one-sided, with the lizards responding to the venom of harvester ants by evolving the capability of neutralizing the ant venom lethality. In contrast, the ants may not have responded directly to horned lizard predation, but might have evolved their ever more lethal venom in response to a common ancestor of horned lizards. Exactly what pressures influenced the evolution of harvester ant venom lethality remain an enticing mystery.

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Appendix 1. Collection locations and dates for *Pogonomyrmex* harvester ants used in this study. All ants were foraging workers collected from around the nest entrance or near the surface within the nest. Locations are listed as country, state abbreviation, county within the state, and location within the county. Dates are listed as time of collection; the ants were then either maintained alive or frozen at −20°C until dissected to obtain their venom. *Pogonomyrmex cunicularius* inhabits only southern South America.

Ant species	Collection location	Date
Pogonomyrmex apache	USA: TX: Lubbock Co.: Lubbock	27 May 1984
P. anzensis	USA: CA: San Diego Co.: Anza-Borrego: Split Mt.	27 April 1998
P. badius	USA: GA: Burke Co.: Waynesboro	22 May 1976
P. barbatus	USA: TX: San Patricio Co.: Welder Refuge	30 October 1979
P. bicolor	USA: AZ: Pima Co.: Tucson: Pima Canyon	14 August 1984
P. brevispinosus	USA: CA: Fresno Co.: Riverdale	28 May 1983
P. californicus	USA: NM: Hidalgo Co.: Skeleton Canyon	24 July 1982
P. comanche	USA: OK: Jackson Co.	25 May 1984
P. desertorum	USA: AZ: Pima Co.: Tucson	27 April 1983
P. huachucanus	USA: AZ: Cochise Co.: Stump Canyon	3 August 1986
P. magnacanthus	USA: CA: Riverside Co: Mecca	6 September 1982
P. maricopa	USA: AZ: Cochise Co.: Willcox	15 March 1983
P montanus	USA: CA: San Bernardino Co.: Fawnskin	3 September 1982
P. occidentalis	USA: AZ: Cochise Co.: Portal	7 June 1981
P. rugosus	USA: AZ: Cochise Co.: Willcox	20 September 1981
P. salinus	USA: OR: Malheur Co.	June 1984
P. salinus owyheei	USA: ID: Nez Perce Co.: Hells Gate	13 June 1984
P. subdentatus	USA: CA: Alameda Co.	4 June1985
P. subnitidus	USA: CA: Riverside Co: San Bernardino Natl. Forest: Vista Grande	5 September 1982
P. tenuispina	Mexico: Baja California Sur: Santiago	13 November 1982
P. texanus	USA: TX: Brewster Co.: Marathon	10 June 1983
P. wheeleri	Mexico: Sinaloa: Mazatlán	6 July 1983
P. cunicularius	Argentina: La Rioja: Chamical	20 August 2000